

Interfacial Properties and Unfolding Equilibria of Globular Proteins

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A thermodynamic study is presented on the influence of interfacial phenomena on protein stability. The main aim of this work was to assess the possibility of stabilizing proteins through increases in the protein-solvent interfacial energy attained by addition of solvent components. Attention was focused on three globular proteins, hen egg lysozyme, *A. niger* glucose oxidase and erythrocyte carbonic anhydrase, differing in size and conformational stability. They were dissolved in aqueous media of different nature and subjected to heating. Aqueous media were prepared by addition of hydroxylic or aminoacidic components at concentrations up to 30% w/w. Ultraviolet difference spectroscopy was used to monitor the conformational changes induced by heating and to determine the melting temperature of the protein. For all the proteins investigated, this quantity was found to be linearly related to the surface tension of the mixed solvent. To clarify the physical bases of this phenomenon, a molecular thermodynamic model was developed. It was based on the two-state mechanism of inactivation, according to which only native folded and polymorphous unfolded protein forms are present at equilibrium. Changes in stability observed in the different media were therefore described in terms of solvent-induced perturbations of the protein distribution between the two conformational states. To express the chemical potential of the protein forms as a function of intensive state properties, an expression was derived for the Gibbs free energy of the system, which accounts for conformational changes, ideal mixing effects, and interaction of the protein with the surrounding medium. As regards the latter term, the Dupré equation was used to relate the interfacial free energy between the protein and the solvent to the free energy of adhesion and to the surface tension of the mixed solvent. Model predictions were in fairly good agreement with experimental unfolding data, supporting the point that the influence of water-miscible additives on protein stability does not extend to any special property of these components, but to their ability to perturb the surface tension of water. The proposed model can be used to correlate unfolding equilibrium data and as a means to approach the problem of protein stabilization by solvent engineering.